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学位授与の要件	課程博士（学位規則第4条第1項）
学位授与の題目	Electrochemical and spectral studies on the reactions of nitric oxide and nitrite with water-soluble metalloporphyrins （水溶性金属ポルフィリンと一酸化窒素および亜硝酸イオンとの反応に関する電気化学的および分光学的研究）
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学位論文要旨

This work presents a study on the series of complexes of *meso*-tetrakis(N-methyl-4-pyridyl)porphyrin (H_2(4-TMPyP)) with Fe(III), Mn(III), Ni(II) and O=V(IV), from the point of their possible catalytic activity towards the electrooxidation of nitric oxide and nitrite.

Chapter 2 presents the study on binding of NO to Fe(4-TMPyP) and its role in the catalytic oxidation of nitric oxide. The reaction of iron(III) (*meso*-tetrakis(N-methylpyridinium-4-yl)porphyrin (Fe^{III}(4-TMPyP)) with nitric oxide (NO) was studied by electronic absorption spectroscopy, ESR, electrochemical and spectroelectrochemical techniques in aqueous solutions with pH from 2.2 to 12.0. Fe^{III}(4-TMPyP) has been found to undergo a reductive nitrosylation (Eq. 1) in all pHs, and the product of nitric oxide binding to the porphyrin has been determined as iron(II) porphyrin nitrosyl complex [Fe^{II}(NO)(4-TMPyP)].

$$[Fe^{III}TMPyP]^{5+} + 2NO + H_2O \rightarrow \quad (1)$$
$$[Fe^{II}(NO)TMPyP]^{4+} + NO_2^- + 2H^+$$

As could be seen from Fig. 1 and Fig. 2, final spectra obtained by bubbling NO into Fe^{III}(4-TMPyP) and Fe^{II}(4-TMPyP) are the same with Soret band at 423 nm and Q band at 552 nm, which suggests the same product, namely Fe^{II}(NO)(4-TMPyP).

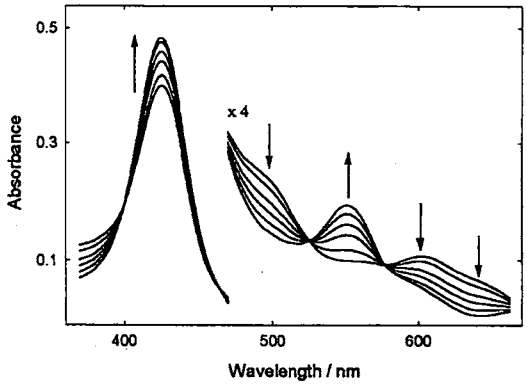


Fig. 1. Electronic absorption spectral changes obtained during 5% NO bubbling into 0.1 mM Fe^{III}(4-TMPyP) in pH 7.4 PBS solution for 2.5 h (every 0.5 h).

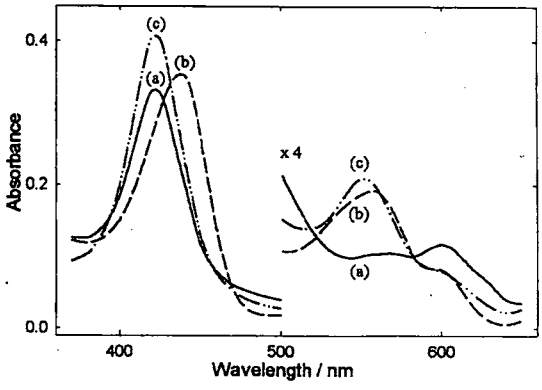


Fig. 2. Electronic absorption spectra of 0.1 mM Fe^{III}(4-TMPyP) in pH 7.4 PBS: (a) before and (b) after electrolysis at –0.4 V; (c) after 5% NO bubbling into electrolyzed solution.

The reductive pathway of nitrosylation has been verified by ESR measurements. The fine structure and g values of the ESR spectrum obtained at 77 K for the Fe(4-TMPyP) adduct with NO (Fig. 3) was very similar to that reported for the product of reductive nitrosylation of hemoglobin. Diamagnetic $[Fe^{III}(NO)(4\text{-}TMPyP)]$ complex would give no ESR signal. Although both $Fe^{III}(4\text{-}TMPyP)$ and NO are paramagnetic species, measurements of their ESR spectra confirmed that neither could be responsible for the spectrum in Fig. 3.

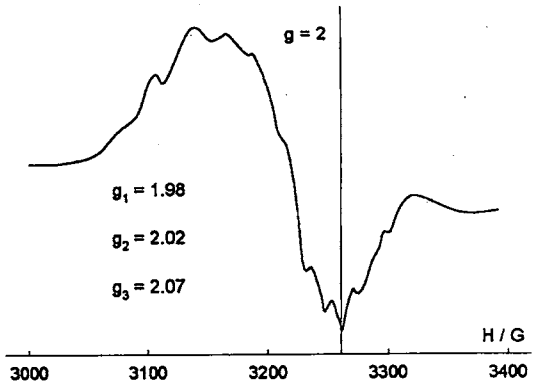
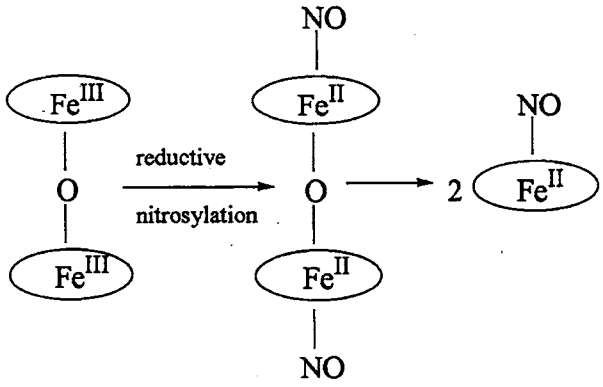


Fig. 3. ESR spectrum of $Fe^{III}(4\text{-}TMPyP)$/NO adduct in pH 7.4 PBS at 77 K.

The rate of the reductive nitrosylation exhibits a tendency to get faster with increase in pH. Intermediate species was observed around neutral pH by spectroelectrochemical technique, and was proposed to be iron(II) nitrosyl complex of μ-oxo dimeric form of Fe(4-TMPyP), which is known to be a predominant in neutral solutions (Scheme 1). Similar intermediate species were observed during electroreduction of $Fe^{III}(4\text{-}TMPyP)$ in solution containing NO.



Scheme 1. Role of μ-oxo dimeric species in reductive nitrosylation.

$[Fe^{II}(NO)(4\text{-}TMPyP)]$ was oxidized at 0.45 V. The product was confirmed to be $Fe^{III}(4\text{-}TMPyP)$ not $[Fe^{III}(NO)(4\text{-}TMPyP)]$, which means that iron(III) nitrosyl complex readily released NO (Eq. 2).

$$[Fe^{II}(NO)TMPyP]^{4+} - e^- \rightarrow$$

$$\{[Fe^{III}(NO)TMPyP]^{5+}\} \rightarrow \quad (2)$$

$$Fe^{III}TMPyP^{5+} + NO$$

The present research demonstrated that $Fe^{III}(4\text{-}TMPyP)$ undergoes reductive nitrosylation in aqueous solutions. From this point of view, behavior of $Fe^{III}(4\text{-}TMPyP)$ is somewhat similar to that of natural hemoproteins. On the other hand, it was different from that of iron *meso*-tetrakis(4-sutfonatophenyl)porphyrin (FeTPPS) – synthetic water-soluble porphyrin often used in model studies – which just bind NO reversibly without reductive nitrosylation. Two different results demonstrate that binding properties of nitric oxide towards iron porphyrins are strongly dependent on the charges of porphyrin peripheries even when rather simple porphyrins are used. Therefore, the result obtained for certain porphyrin should be used very carefully when applied to another one in order not to make hasty conclusions. This could be especially misleading when simplified model porphyrins are exploited instead of natural species.

Charged side groups in the porphyrin structure seem to have a prevalent effect on the nitrosylation reaction. It should be noted that expressing NO ligand in nitrosyl complexes as uncharged group, is quite a formal. Electron transfer between iron cation and nitrosyl group is strongly pronounced in iron porphyrin nitrosyl complexes. Thus, iron(III) nitrosyl porphyrins could be described by $Fe^{II}(NO^+)P$ structure (P denotes porphyrin), while iron(II) forms have $Fe^{III}(NO^-)P$ structure in some extent. Comparing the nitrosyl complexes of Fe(4-TMPyP) and FeTPPS, it is expected that positively charged peripheries of the former stabilize nitroxyl anion and iron(II) nitrosyl complex, consequently, but negative charges on FeTPPS have an opposite effect. This is

one of probable reasons why reductive nitrosylation was not observed in the case of FeTPPS. On the other hand, electrophilic properties of nitric oxide coordinated to iron(III) porphyrin should play an important role in reductive nitrosylation. Quarternized pyridyl-moieties with high electron-withdrawing ability make nitrosonium ion in $[Fe^{II}(NO^{+})(4\text{-}tMPyP)]^{5+}$ extremely electrophilic and reaction with nucleophiles like water or hydroxyl anion easy. Negatively charged sulfonato-groups in $[Fe^{II}(NO^{+})TPPS]^{3-}$, in contrast, might protect nitrosyl from nucleophilic attack and reductive nitrosylation, therefore. Summarizing our results, an assumption could be done that reductive nitrosylation is a general feature for natural and synthetic iron(III) porphyrins in aqueous solutions. The ability of certain porphyrin to react in this way, however, depends on its structure namely electronic properties of the porphyrin side groups.

Chapter 3 explains a catalytic behavior of Mn(4-TMPyP) towards electrooxidation of NO and nitrite. Catalytic oxidation of nitric oxide and nitrite by water-soluble manganese(III) meso-tetrakis(N-methylpyridinium-4-yl)porphyrin was first studied at an indium-tin oxide (ITO) electrode in pH 7.4 phosphate buffer solutions. A stepwise oxidation of Mn^{III}(4-TMPyP) through high-valent O=Mn^{IV}(4-TMPyP) and O=Mn^{V}(4-TMPyP) porphyrin species has been observed by electrochemical and spectroelectrochemical (OTTLE) techniques (Scheme 2).

The formal potential of 0.634 V for the first oxidation step has been estimated from OTTLE data (Fig. 4). OxoMn(IV) porphyrin, was relatively stable and decayed slowly to Mn^{III}(4-TMPyP) with a first-order rate constant of 3.7×10^{-3} s^{-1}.

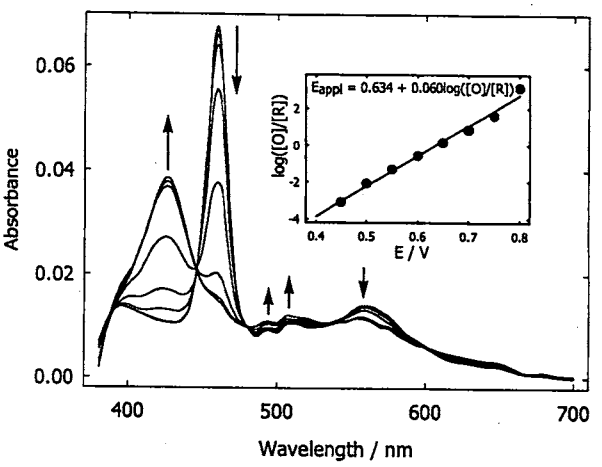


Fig. 4. Electronic absorption spectral change obtained in pH 7.4 PBS solution containing 20 μM Mn(4-TMPyP) by applying different potentials in range from 0.4 to 0.8 V (each curve corresponds to 50 mV step). Inset: plot of log([O]/[R]) vs. E_{appl}.

O=Mn^{IV}(4-TMPyP) has been found to oxidize NO catalytically at potentials about 0.9 V (Fig. 5, curve f) with good selectivity against nitrite. Nitrite was catalytically oxidized at potentials higher than 1.1 V by O=Mn^{V}(4-TMPyP) (Fig. 5, curve d). Product analysis by Griess method confirmed that nitrate was the final product of the electrolysis at 1.2 V, while at 0.8 V nitrite left unchanged, demonstrating that O=Mn^{IV}(4-TMPyP) could not oxidize nitrite.

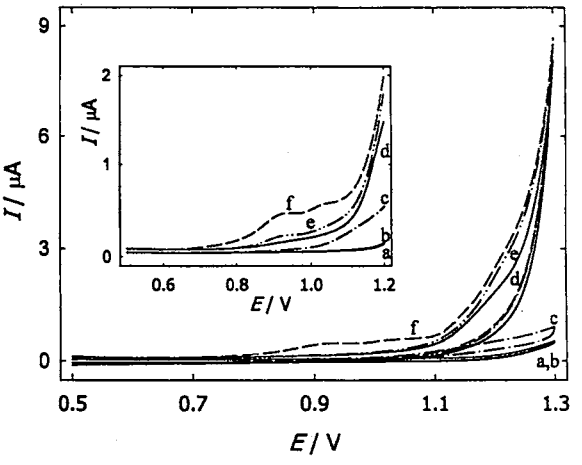
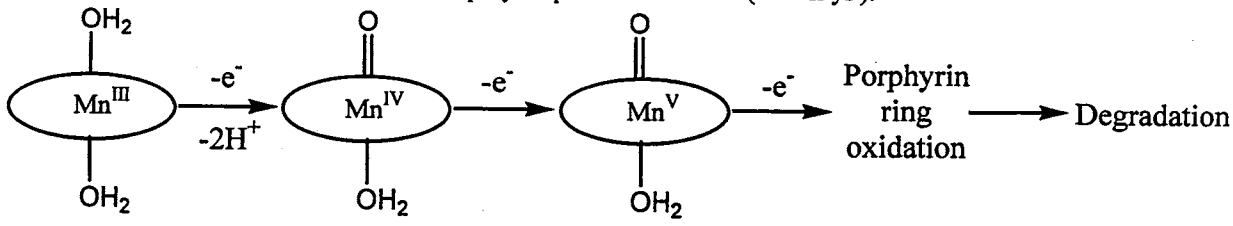
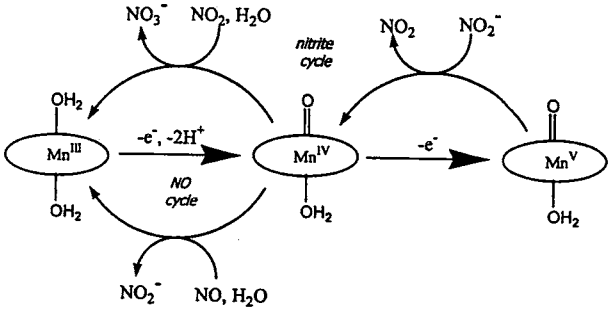


Fig. 5. Cyclic voltammograms obtained at an ITO electrode in pH 7.4 PBS solution: (a) without and (b) with 20 μM nitrite, and (c) with 20 μM nitrite and 20 μM NO. Other curves were measured in PBS solution containing 10 μM Mn(4-TMPyP): (d) without and (e) with 20 μM nitrite, and (f) with 20 μM nitrite and 20 μM NO. Scan rate, 10 mVs^{-1}. Inset: cyclic voltammograms (only anodic halves shown) obtained under the same conditions in shorter potential range for more precise representation of the first oxidation step.

Scheme 2. Step-by-step oxidation of Mn(4-TMPyP).



A possible schemes of the catalytic oxidation of NO by $O=Mn^{IV}$(4-TMPyP) and NO_2^- by $O=Mn^{V}$(4-TMPyP) have been proposed. The maximum catalytic current of the oxidation of nitrite by Mn(4-TMPyP) observed at 1.25 V was similar to that obtained using Fe(4-TMPyP) at 1.12 V. It means iron porphyrin is better catalyst for the oxidation of nitrite, because it allows oxidizing at more negative potentials. On the other hand, manganese porphyrin can oxidize nitric oxide at potentials of about 70 mV more negative than iron porphyrin does. A potential gap between the oxidation of NO and NO_2^-, therefore, is greater in the case of Mn(4-TMPyP). It makes manganese porphyrin more selective catalyst for NO oxidation comparing with iron porphyrin.



Scheme 3. A possible reaction mechanism for the catalytic oxidation of NO and nitrite by high-valent manganese porphyrin.

Chapter 4 shows the results of the comparative study on the electrooxidation of nitrite mediated by different metal complexes of H_2TMPyP, namely Fe(III)-, Mn(III)-, Ni(II)- and O=V(IV) porphyrins. Cyclic voltammetric studies show that all porphyrins can catalyze the oxidation of nitrite at potentials that correspond to the porphyrin ring oxidation. The oxidation potential grows in the series Ni < metal-free < Fe < V < Mn, which comes to a good agreement with the known effect of metal cation electronegativity on the porphyrin ring oxidation potential. Maximum catalytic currents are roughly the same for all metal porphyrins suggesting similar nature of catalysis in all cases (Fig. 6), which supports the idea of porphyrin π-cation radical involvement in this process. At the same time, metal-free H_2(4-TMPyP) was not as good catalyst as its metal complexes. The role of the metal cation in the catalysis, therefore, seems to be not just limited to the regulation of the porphyrin ring oxidation potential. Metal site may participate in the stabilization of the reaction intermediates.

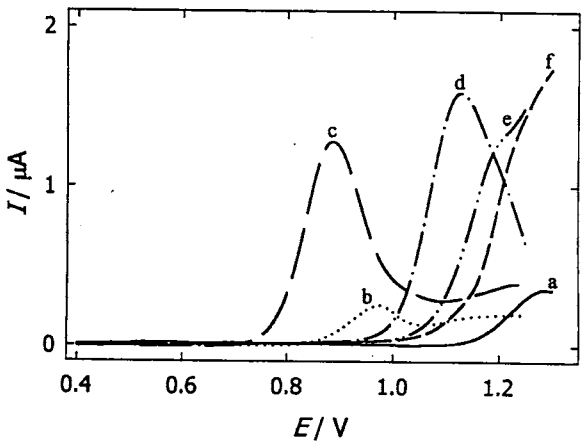


Fig. 6. Cyclic voltammograms (only anodic halves shown) of the catalytic oxidation of 50 μM of nitrite at an ITO electrode in pH 7.4 PBS containing 10 μM of the following porphyrins: (a) pure PBS, (b) H_2(4-TMPyP), (c) Ni(4-TMPyP), (d) Fe(4-TMPyP), (e) O=V(4-TMPyP) and (f) Mn(4-TMPyP. Scan rate, 10 mV s^{-1}.

Iron and manganese porphyrins catalyze electrooxidation of NO at potentials where these two porphyrins undergo an oxidation at their metal site, while nitrite is oxidized along with the porphyrin ring oxidation. Other porphyrins catalyze oxidation of both NO and nitrite at similar potentials without making any discrimination between these two species and showing no any selectivity,

therefore. This is a key point of the obtained results that some porphyrins by themselves can introduce some selectivity to NO detection. Therefore, sensors based on iron and manganese porphyrins could be more promising for the detection of NO than a traditional Ni-porphyrin sensor.

学位論文審査結果の要旨

本学位論文に関して、平成17年2月2日の口頭発表後、論文審査委員会を開催し、審議の結果、以下のとおり判定した。

本論文は、電気化学的手法により生体中の微量一酸化窒素を選択的に検出するために必要な要素の解明を目的とし、鉄、マンガン、ニッケル、バナジルなどの種々の水溶性金属ポルフィリン[M（4-TMPyP）]を用いて得た成果をまとめたものである。すなわち鉄ポルフィリン[Fe（4-TMPyP）]は一酸化窒素の存在下、還元的ニトロシル化を受けることをはじめて見い出し、反応機構の詳細を明らかにした。また、マンガンポルフィリン[Mn（4-TMPyP）]は電位によって、異なる高原子価錯体を生成し、オキソマンガン(IV)錯体は一酸化窒素のみの酸化を、オキソマンガン(IV)π-カチオンラジカル錯体は亜硝酸イオンの酸化を触媒する機構を、ITO（Indium-Tin Oxide）電極を用いて明らかにした。これにより、妨害物質である亜硝酸イオンが共存していても電位をコントロールすることにより一酸化窒素の選択的検出が可能となることを指摘した。一方、優れた一酸化窒素検出用の触媒とされてきたニッケルポルフィリン[Ni（4-TMPyP）]は一酸化窒素と亜硝酸イオンを区別して検出することが不可能であることを明らかにした。

以上のように本論文は関連分野の発展に大きく貢献する成果を挙げていることから、博士(学術)の学位を与えるに値するものと考え、審査員一致で合格と判定した。